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Toxicity of Bromate to Striped Bass Ichthyoplankton ('Morone saxatilis') and Juvenile Spot ('Leiostomus xanthurus')

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16. ABSTRACT
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TOXICITY OF BROMATE TO STRIPED BASS ICHTHYOPLANKTON (*MORONE SAXATILIS*) AND JUVENILE SPOT (*LEIOSTOMUS XANTHURUS*)

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*Striped bass (*Morone saxatilis*) eggs (12 h after fertilization) and larvae (4 d after hatching) and juvenile spot (*Leiostomus xanthurus*) were exposed to a series of bromate concentrations for 4, 10, and 10 d, respectively, using static replacement bioassay techniques. Three-dimensional mortality response surfaces were constructed by computerized probit regression techniques. Newly hatched striped bass prolarvae were most sensitive to bromate and had a 96-h LC50 of 30.8 mg/l (as BrO_3^-). Four-day-old striped bass larvae were less sensitive, with 2- to 10-d LC50s ranging from 605.0 to 92.6 mg/l BrO_3^-, respectively. Juvenile spot were least sensitive, with 1- to 10-d LC50s ranging from 698.0 to 278.6 mg/l BrO_3^-, respectively.*

INTRODUCTION

Bromate (BrO_3^-) has been identified as a stable end-product of exposure of chlorinated seawater to sunlight (Macalady et al., 1977). Crecelius (1979) found that O_3, another strong oxidant that has been suggested as a possible substitute for chlorine, produces bromate in seawater without sunlight. Large quantities of estuarine and marine water are treated with chlorine to prevent fouling of the condenser systems at power plants, and a variety of

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waste treatment processes discharge chlorinated effluents into estuarine and marine environments. Both O_3 and chlorine form bromate through a complicated series of reactions with the Br^- that is naturally present in estuarine and marine waters (Richardson et al., 1981). Bromate is very stable once formed, with no detectable decay for periods up to 1 mo. Thus large amounts of bromate could be produced in the environment, and there is little information concerning its toxicity to estuarine and marine organisms.

Crecelius (1979) conducted preliminary bioassays to determine the toxicity of bromate to several marine organisms and found a relatively low acute toxicity with LC50 values up to several hundred milligrams per liter. However, 30 mg/l BrO_3^- caused abnormal development in 50% of his Pacific oyster larvae (*Crassostrea gigas*) during the first 48 h of larval development. In contrast, Stewart et al. (1979) found that only 1.0 mg/l BrO_3^- caused 90% mortality in the American oyster (*Crassostrea virginica*). Because of the sparsity of information on bromate toxicity to marine organisms, we conducted the present study to determine the toxicity of bromate to selected estuarine ichthyoplankton and juvenile fish. Striped bass (*Morone saxatilis*) eggs, prolarvae, and larvae and juvenile spot (*Leiostomus xanthurus*) were studied because of their importance in many eastern and Gulf Coast estuaries.

MATERIALS AND METHODS

Striped Bass Eggs and Larvae

Fertilized striped bass eggs (water-hardened) were obtained from the Brookneal Striped Bass Hatchery (Brookneal, Va.) and transported to the laboratory in oxygen-filled plastic bags, which were placed in Styrofoam chests to maintain a constant temperature of ≃20°C. Eggs were transferred to standard 6-l hatchery jars supplied with a continuous flow of filtered Patuxent River water (≃20°C and 5 ppt salinity); all dead and unfertilized eggs were allowed to purge from the stock jars. Experiments were begun with eggs that were 12 h old (measured from time of fertilization), using standard, static renewal bioassay procedures (American Public Health Association, 1976).

For the egg-prolarvae experiment 40 striped bass eggs were placed in 1-l beakers containing 0, 10, 20, 50, 75, 100, 200, 300, and 500 mg/l BrO_3^-. Test solutions were made with filtered Patuxent River water (≃5 ppt and reagent grade sodium bromate; solutions were changed every 24 h. Controlled, artificial oxygenation was performed continuously throughout the egg and larvae studies to ensure adequate dissolved oxygen (DO). Aeration and handling during solution changes were performed with minimal agitation of the eggs or prolarvae, although continuous agitation of eggs is standard hatchery procedure.

The 12-h-old eggs were allowed to develop through hatch, which occurred approximately 26 h into the study (38 h postfertilization: PF); percent hatch was estimated after 36 h (48 h PF) of exposure. Total egg and/or prolarval mortality was assessed at each concentration after 24, 36, 58, 72, and 96 h of continuous exposure. Thus the egg stage received approximately 26 h exposure to the bromate concentrations, while prolarvae hatched from exposed eggs received approximately 70 h total exposure.

For the larval experiment eggs were reared in the laboratory under continuous-flow conditions, using filtered Patuxent River water (≃5 ppt), until 96 h posthatch (PH). Twenty larvae were tested in each of nine 1-l beakers containing bromate in the same concentrations used in the egg study. Mortality observations were made every 24 h for 10 d and bromate solutions were changed every 24 h. Larvae were fed fresh *Artemia* nauplii once each day after they had begun active feeding (4 to 6 d PH). All test beakers were maintained at approximately 20°C in a constant-temperature water bath throughout the 10-d study.

Juvenile Spot

Juvenile spot were collected at the Calvert Cliffs nuclear power plant on the Chesapeake Bay in Calvert County, Md. Fish were transported to the laboratory, where they were acclimatized for at least 4 wk in 800-l fiberglass holding tanks supplied with a continuous flow of Patuxent River water. All fish were fed twice daily with precooked commercial shrimp and exposed to a 12-h light, 12-h dark photoperiod during acclimation and testing.

Groups of 15 spot, 92.9 ± 1.80 mm (total length) and 7.9 ± 0.57 g (wet weight), were placed in standard 46-l all-glass aquariums containing 50, 75, 100, 180, 240, 320, 385, 470, 560, and 1000 mg/l BrO_3^- for 10 d. Mortality observations and water quality measurements were made every 12 h; test solutions were changed every 24 h. Controlled artificial oxygenation was performed continuously throughout the study.

Water quality values (mean ±SD) during acclimation and testing for all studies were: temperature, 20.1 ±0.25°C; salinity, 5.1 ± 0.10 ppt; pH 7.8 ± 0.03; DO, 7.0 ± 0.09 mg/l; and ammonia N, 1.0 ± 0.08 mg/l.

Bromate Analysis

Bromate concentrations were analyzed by differential pulse polarography on a Princeton Applied Research (PAR) polarographic analyzer (model 174A; Princeton Applied Research Corp., Princeton, N.J.) connected to an X-Y recorder (model 7040A; Hewlett-Packard, San Diego, Calif.). Polarograms were recorded at the sample temperature of 20°C after 3 min of oxygen stripping with high-purity nitrogen in an analytical cell equipped with a PAR dropping mercury electrode and a saturated calomel reference electrode. No major differences were found between measured and calculated bromate concentrations.

Statistical Analysis

Data for all experiments were analyzed with a computer probit regression program (SAS Institute, 1979). Probit regressions were performed stepwise for each exposure period and combined graphically to yield time × concentration × mortality response surfaces. A test of goodness of fit for the response surfaces was derived by combining the individual chi-square values from each probit regression, as allowed by the reproductive property of the chi-square distribution (Beyer, 1971). During the egg-prolarvae study, the hatchability of the striped bass eggs was assessed by computing the percentage of successfully hatched eggs for each bromate concentration approximately 36 h into the experiment (48 h PF). For a complete discussion of the probit techniques see Finney (1971).

RESULTS AND DISCUSSION

Striped Bass Eggs and Larvae

Striped bass eggs (12 h PF) exposed to 0, 10, 20, 50, 75, 100, 200, 300, and 500 mg/l BrO_3^- for a maximum of 36 h had hatch percentages of 95, 90, 80, 85, 88, 73, 83, 58, and 58, respectively. A progressively longer delay in hatching time was observed as bromate concentrations increased above 100 mg/l, with the greatest delay ($\simeq$10 h) occurring at 500 mg/l. The hatch time-temperature relation for striped bass eggs has been given by Rogers et al. (1977) as

$$T = 258.5\, e^{-0.0934(t)}$$

where T is time (h) from fertilization to hatch and t is water temperature (°C). This relation gives an expected hatch time of $\simeq$40 h for eggs raised at 20°C, which closely agrees with the $\simeq$38 h from fertilization to hatch observed in our control. At bromate concentrations ($\geqslant$100 mg/l) where delayed hatching was observed, all eggs that had not hatched within 10 h of the controls died without hatching.

Mortality data for the egg-prolarvae study are shown in Table 1 and Fig. 1. The response surface was predictable statistically at the 0.05 level. Con-

TABLE 1. Toxicity of Bromate to Eggs and Prolarvae of the Striped Bass (*Morone saxatilis*)

Exposure time (h)	LC50 (mg/l as BrO_3^-)	95% confidence limits
24	697.4	313.0–5974.9
Hatch (26–36 h)	—	—
36	103.2	74.5– 139.2
58	42.9	30.0– 56.9
72	39.8	27.5– 51.4
96	30.8	20.5– 40.2

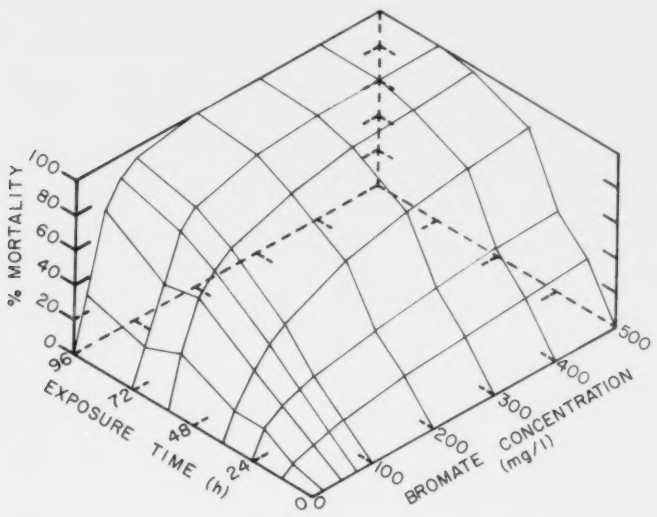

FIGURE 1. Percent mortality response surface for egg-prolarvae stages of striped bass exposed to bromate.

fidence limits for the 24-h exposure data (Table 1) were exceptionally large, since the LC50 was derived by extrapolation beyond the range of concentrations tested. Prolarvae were much more sensitive to bromate than eggs, as indicated by the large decrease in LC50 following hatch. This increased sensitivity is probably due to the absence of a chorion, which acts as a protective covering for the egg during the early stages of development (Middaugh et al., 1978).

Newly hatched prolarvae in concentrations above 75 mg/l (i.e., 100–500 mg/l) exhibited progressively greater larval deformation with increasing concentration (qualitative observation). Deformed larvae appeared to be smaller than control larvae, most had various degrees of spinal curvature, and a few in the highest concentrations had enlarged forebrain areas (pugheadedness). Similar physical abnormalities have been observed for striped bass eggs exposed to severe thermal shock (Koo and Johnston, 1978) and for striped bass and blueback herring eggs and larvae exposed to residual chlorine (Morgan and Prince, 1977, 1978).

The 10-d experiment with striped bass larvae that were initially 4 d old (i.e., 4 d pH) was conducted to determine whether older larvae are more sensitive to bromate than young prolarvae. The results of this experiment are shown in Table 2 and Fig. 2. The combined chi-square analysis for the response surface in Fig. 2 showed that the model accurately represents the data at the 99% confidence level. The 1-d mortality data in Fig. 2 are the actual values, since there was only minimal mortality (10%) at the highest concentration and thus not enough data to be analyzed statistically.

TABLE 2. Toxicity of Bromate to 4-d-old Striped Bass Larvae (*Morone saxatilis*)

Exposure time (d)	LC50 (mg/l as BrO_3^-)	95% confidence limits
1	–	–
2	605.0	507.0–1007.3
3	491.7	438.9– 621.4
4	403.5	350.3– 480.4
5	313.4	272.8– 366.4
6	230.3	199.9– 271.0
7	197.2	172.3– 229.1
8	177.4	147.5– 210.0
9	148.3	114.2– 185.0
10	92.6	–[a]

[a]Limit undefined by computer calculations.

Comparison of the egg-prolarvae and larval experiments shows that eggs are relatively resistant to bromate, while newly hatched prolarvae are more sensitive but become more resistant within a few days. Age-related sensitivity was also noted by Alderson (1972, 1974) for mortality of plaice (*Pleuronectes platessa* L.) and Dover sole (*Solea solea* L.) exposed to chlorinated seawater. He found that eggs were less sensitive than newly hatched larvae and that larval sensitivity decreased with increasing age up to metamorphosis. Other investigators have shown that larvae of striped bass and

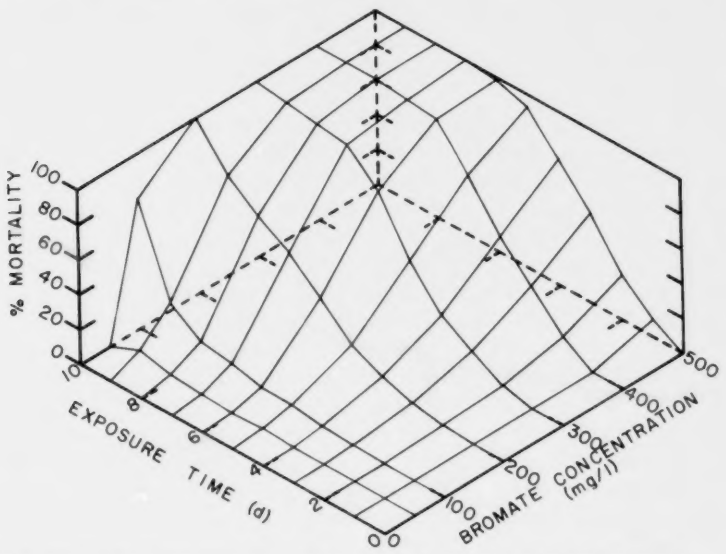

FIGURE 2. Percent mortality response surface for striped bass larvae exposed to bromate.

other estuarine and marine species are more sensitive than eggs when exposed to chlorinated waters (Morgan and Prince, 1977; Middaugh et al., 1977, 1978; Burton et al., 1979a, 1979b).

Juvenile Spot

The toxicity of bromate to juvenile spot is shown in Table 3. LC50 values were derived from stepwise (by day) probit regression analyses, which showed significant heterogeneity for the data between d 1 and 7 inclusive. Thus the LC50 values for d 1-7 represent a trend and should not be viewed as well-defined limits. Data for d 8-10 were not heterogeneous and represent accurate mortality predictions within the indicated limits. A mortality response surface (Fig. 3) was constructed by combining the individual probit regression equations to graphically show bromate toxicity to juvenile spot.

The data obtained for spot are consistent with the findings of Crecelius (1979), the only other author, to our knowledge, who has conducted bromate toxicity experiments with marine or estuarine fish. He found a 96-h LC50 of 512 mg/l (as BrO_3^-) for the toxicity of bromate to juvenile chum salmon (*Oncorhynchus keta*). This value is of the same order of magnitude as that derived from our experiments with juvenile spot, although a quantitative comparison cannot be made because of the heterogeneous nature of our data.

CONCLUSIONS

The results of this study show that newly hatched striped bass prolarvae are most sensitive to bromate, followed by older larvae, eggs, and juvenile spot. In contrast, the early developmental stages of some molluscan larvae

TABLE 3. Toxicity of Bromate to Juvenile Spot (*Leiostomus xanthurus*)

Exposure time (d)	LC50 (mg/l as BrO_3^-)	95% confidence limits
1	698.0[a]	492.9-1946.2
2	517.6[a]	368.1- —[b]
3	468.2[a]	267.6- 765.3
4	427.4[a]	322.4- 685.4
5	415.2[a]	322.6- 604.7
6	398.9[a]	304.1- 590.3
7	378.9[a]	293.6- 527.7
8	337.2	295.4- 387.1
9	295.8	258.4- 337.4
10	278.6	243.9- 316.9

[a]Significant heterogeneity.
[b]Limit undefined by computer calculations.

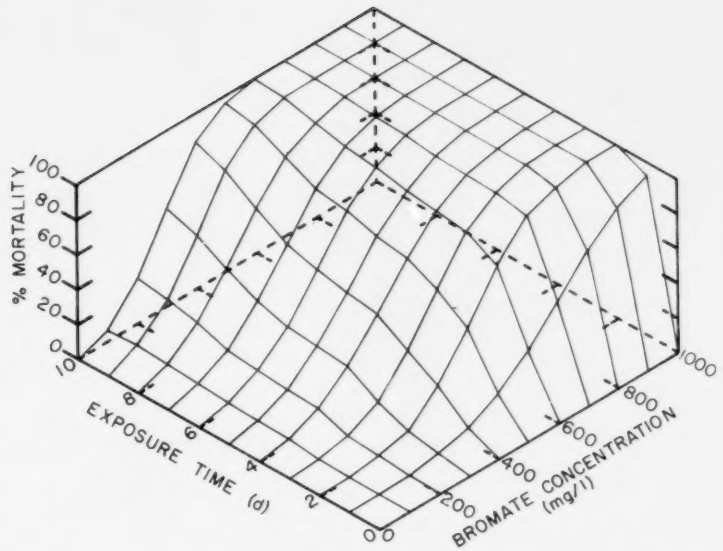

FIGURE 3. Percent mortality response surface for juvenile spot exposed to bromate.

appear to be an order of magnitude more sensitive than other invertebrates, ichthyoplankton, and juvenile and adult fish (Crecelius, 1979; Stewart et al., 1979). Furthermore, Crecelius (1979) and Macalady et al. (1977) showed in the laboratory that bromate can be formed in chlorinated or ozonated seawater at concentrations that could have a deleterious effect on American oyster larvae (Stewart et al., 1979). Thus bromate concentrations produced by chlorination or ozonation of natural estuarine or marine water could be high enough to affect some molluscan larvae but would probably have little or no acute effect on other invertebrates, ichthyoplankton, and juvenile and adult fish. Sublethal effects of bromate have not been investigated and may be significant for extended exposure periods. We suggest that studies be conducted in the field to determine the amount of bromate that could occur in chlorinated or ozonated estuarine and marine discharges. Additional toxicity studies on the early developmental stages of mollusks are also needed to verify the potential detrimental effects observed by Stewart et al. (1979).

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